

# Composition Studies on Tobacco. XXVII. Polyphenol-Amino Acid Leaf Pigment: Further Structural Investigations

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The presence of a high molecular weight pigment in cigarette smoke was reported earlier (1, 10). This pigment was superficially similar to the iron-polyphenol-amino acid pigment found in leaf (13, 5, 2). Among other differences, the smoke pigment had a higher proportion of linkages resistant to alkaline and acidic hydrolyses, as indicated by the relative yields of quinic and amino acids. Thus, the bulk of the structure appeared to consist of either unhydrolyzable chlorogenic and amino acids or moieties structurally unrelated to such compounds. More recent work (3) showed that, in addition to the above components, several alkaloids and a silicone were linked within the pigment structure. As far as we know, this was the first report of the occurrence in smoke of nicotine and related bases bound in a high molecular weight substance. This finding prompted a further investigation of the structure of the leaf pigment.

## Method

Smoke pigment was isolated from the condensate of domestic, commercial cigarettes, and a nondialyzable, ether-insoluble weakly acidic fraction of the pigment was obtained by methods previously described (3, 10). Leaf pigment was isolated from Turkish tobacco by the method outlined earlier (2). The crude pigment was dialyzed against running tap water for 30 hours followed by dis-

tilled water for 2 days, and the nondialyzable fraction was extracted continuously with chloroform for 5 days. The nondialyzable, chloroform-insoluble subfraction of the pigment was used in this study.

Alkaline fusions were conducted by the general procedure described previously (3). In all cases, the fusion was performed under nitrogen and pressure (50 lbs.) until the final reaction temperature (260°-280°C) was reached (about 30 mins.) at which time the pressure was reduced to ambient, the temperature was maintained and the reaction was continued for 1 hr. The bases in the reaction mixture were isolated as the hydrochlorides and identified by the thin layer and gas chromatographic methods outlined elsewhere (3, 9). Comparisons of the relative amounts of components were based on measurement of gas chromatographic peak heights in most cases. When peaks eluted very rapidly or very slowly, making such measurements grossly misleading, peak areas were employed.

The silicone was also isolated and identified from the fusion mixture by the method previously noted (3).

## Results and Discussion

Differences in elemental analyses, iron content and hydrolytic products of the fractions of leaf and smoke pigments have been described (10). In this earlier work, alkaline fusion of the pigments was employed to

demonstrate the presence of phenolic compounds formed in short-term reactions, i.e. 200°-300°C, for up to 5 mins. (10, 13). Such findings were used to confirm the presence of rutin and/or chlorogenic acid therein. In later work (3) and in the present study, longer reaction times were employed to effect more extensive degradation.

Alkaline fusion of the leaf pigment as described above resulted in the release of several bases from the nondialyzable fraction of the leaf pigment (LP). Qualitatively, the basic constituents were somewhat similar to those obtained by identical treatment of the nondialyzable, weakly acidic fraction of the smoke pigment (SP). Table 1 lists the alkaloids and other bases found in LP and SP after a single alkaline fusion. In addition, seven (LP) to fourteen (SP) unidentified components were found in the basic fractions from the fusions. A few bases in Table 1 not identified in the previous report (3) on SP have now been characterized: 2,4-lutidine; 2,4,6-collidine (or 2,3-lutidine); pyridine-3-aldehyde; methyl pyridyl ketone; nicotinonitrile; and dihydro-metanicotine. The major qualitative difference between LP and SP concerned alkaloids in the fusion products. In SP, normicotine, metanicotine and cotinine were present in relatively large amounts. In LP, they were absent or, at the most, present in trace amounts relative to nicotine.

At least two major points are evident from the qualitative findings: the structure of the leaf pigment is more complex than originally believed, and biosynthesis of the leaf pigment must involve reactions other than the polyphenol-amino acid condensations ("browning reactions") previously postulated.

A precise quantitative comparison of the pigments is more difficult since the reproducibilities of the alkaline fusion and subsequent analysis of products have not been investigated fully. However, it appears that a valid comparison can be made of the relative proportions of simple bases (picolines, lutidines, pyrrole, etc.) to nicotine released by LP and SP under identical conditions of fusion. Analytical errors should be at a minimum in such determinations since gas chromatographic peaks from a single injection are compared. Table 1 gives representative data on this point and shows that SP contains larger proportions of simple bases to nicotine. On single alkaline fusion, the absolute nicotine levels released from the pigments were somewhat similar (0.006-0.01%) with some tendency toward a higher level in LP.

Approximately 80% of SP and 20% of LP can be recovered from the fusion mixtures by acidification and filtration in the described manner. In both cases, the recovered material is reduced in molecular weight. For LP, the reduction is from about 16,000-30,000 (unfused pigment) to <4000; for SP, the reduction (3) is from  $\geq 100,000$  to 30,000-100,000. In the case of SP, refusion of the recovered material results in release of a further quantity of nicotine roughly equivalent to that obtained in the first fusion; however, with the exception of pyrrole, essentially no simple bases are obtained by refusion. Thus, the simple bases are almost completely released by a single fusion and appear to be less strongly linked than nicotine in the smoke pigment.

All of these data bear on the question of the origin of SP. An earlier postulation (10, 3) suggested that LP is mechanically transferred into the smoke and undergoes structural alteration, including polymerization in the transition. (Polymerization was suggested to account for the larger molecular weight in the smoke pigment.) The data in Table 1 support this concept. The transition from leaf to smoke results in an increase in the proportion of simple bases to nicotine released by alkaline fusion. Since most of these simple bases

**Table 1. Alkaline fusion products of leaf and smoke pigments**

	Relative amounts*	
	Leaf	Smoke
Pyrrole	.23	5.7
N-Methylpyrrole	.03	A
3-Pyrroline	.19	1.3
Pyrrolidine	.05	1.1
N-Methylpyrrolidine	.02	2.2
Piperidine	A	1.9
Pyridine	.50	2.0
2-Picoline	.10	1.4
3-Picoline	.42	3.9
2,4-Lutidine	.11	1.6
2,6-Lutidine	.03	.61
3,5-Lutidine	.13	.86
2,4,6-Collidine (or 2,3-lutidine)	.10	1.1
3-Vinylpyridine	.03	2.6
3-Ethylpyridine	.06	1.5
Pyridine-3-aldehyde	.15	.64
Methyl-3-pyridyl ketone	.54	3.9
Nicotinonitrile	.13	3.7
Nicotine	1.0	1.0
Nornicotine	A	3.2
Metanicotine	A	4.1
Cotinine	A	2.3
Dihydropyridine	A	.73

\*Nicotine = 1.0 A = compound absent or in trace amounts

are common smoke constituents (9), adequate opportunity for reaction between the bases and the mechanically transferred pigment is available during aerosol formation.

The presence in LP of moieties which are known pyrolytic products of nicotine (e.g., 3-vinylpyridine and pyridines substituted in positions other than 3-) is unexpected. As far as we are aware, 2- and 4-alkyl and related pyridines are not found in tobacco leaf and are not intermediates in established biochemical pathways of alkaloidal synthesis. However, on the basis of recent work, it appears that biochemical transformations of piperidine derivatives into 2-pyridyl derivatives may occur in tobacco leaf (11). The occurrence of more complex 2-pyridyl compounds in leaf is known, i.e. 2,3'-bipyridyl (6), 2,2'-bipyridyl and nicotelline [2,4-di(3-pyridyl)pyridine] (6, 7, 12). The isolation of 2- and 4-substituted pyridines from the alkaline fusion mixture of the leaf pigment is apparently not due to rearrangement or other reactions of nicotine itself during alkaline fusion. On fusion of pure nicotine under identical conditions, none of the

above bases are formed and almost all of the nicotine (> 95%) is recovered unchanged.

A silicone was also isolated in small amounts from the alkaline fusion mixture of LP. The properties of this silicone were similar to the silicone obtained from SP, which was identified as a polymeric methylsiloxane by infrared and mass spectral characteristics (3). A similar substance was reported earlier in cigar smoke (8). As discussed previously (3) the silicone in SP is not a contaminant since it is ether-soluble and should have been removed in the prolonged ether extraction prior to dialysis. If such was not the case, subsequent obstacles in the isolation (solubility in dilute pH 10.0 buffer and dialysis) make the possibility of contamination very remote. The occurrence of organic silicon compounds in plants is known and knowledge in this field has been reviewed recently (4).

#### Summary

The nondialyzable fraction of the leaf pigment previously believed to consist of iron, polyphenols and amino acids has been shown also to contain alkaloids and related

bases and a silicone. Data were obtained which substantiate the concept that the recently isolated smoke pigment is formed during the burning process from the leaf pigment by structural alteration, including reaction with smoke bases.

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